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Triacylglycerol and Phospholipid Fatty Acids of the Silverleaf Whitefly: Composition and Biosynthesis

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The identification and composition of the fatty acids of the major lipid classes (triacylglycerols and phospholipids) within *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) nymphs were determined. Comparisons were made to fatty acids from the internal lipids of *B. argentifolii* adults. The fatty acids, as ester derivatives, were analyzed by capillary gas chromatography (CGC) and CGC-mass spectrometry (MS). All lipid classes contained variable distributions of eight fatty acids: the saturated fatty acids, myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), arachidic acid (20:0); the monounsaturated fatty acids, palmitoleic acid (16:1), oleic acid (18:1); the polyunsaturated fatty acids, linoleic acid (18:2), linolenic acid (18:3). Fourth instar nymphs had 5–10 times the quantities of fatty acids as compared to third instar nymphs and 1–3 times the quantities from adults. The fatty acid quantity differences between fourth and third instar nymphs were related to their size and weight differences. The percentage compositions for fatty acids from each lipid class were the same for the pooled groups of third and fourth instar nymphs. For nymphs and adults, triacylglycerols were the major source of fatty acids, with 18:1 and 16:0 acids as major components and the majority of the polyunsaturated fatty acids, 18:2 and 18:3 were present in the two phospholipid fractions, phosphatidylethanolamine and phosphatidylcholine. Evidence was obtained that whiteflies indeed synthesize linoleic acid and linolenic acid de novo: radiolabel from [2-^{14}C] acetate was incorporated into 18:2 and 18:3 fatty acids of *B. argentifolii* adults and CGC-MS of pyrrolidide derivatives established double bonds in the $\Delta^{9,12}$ and $\Delta^{9,12,15}$ positions, respectively. Arch. Insect Biochem. Physiol. 53:66–79, 2003. Published 2003 Wiley-Liss, Inc.†

KEYWORDS: Silverleaf whitefly; nymph; adult; linoleic acid

INTRODUCTION

The silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring [=sweetpotato whitefly, *Bemisia tabaci* (Gennadius), Biotype B], is considered a serious pest of agricultural crops in the southern United States. One of the effective means to control these whiteflies in the field is the use of beneficial predators and parasitoids. The survival and proliferation of these natural enemies of whiteflies are dependent upon the quality and quantity of the nutritional potential of whitefly eggs and nymphs. Predators of whiteflies consume eggs and/or nymphs, whereas specific parasitoids (parasitic wasps) usually prey upon the nymphal stages (Gerling, 1990). One of the essential nutritional needs for predacious insects is lipid (Cohen, 1995). Homoptera (whiteflies, aphids, scale insects) are

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Abbreviations used: FAME = fatty acid methyl ester; FFA = free fatty acid; PC = phosphatidylcholine; PE = phosphatidylethanolamine; TAG = triacylglycerol; *t*-BME = *tert*-butylmethylether.

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generally phloem feeders and plant phloem sap contains slight, if any, amounts of lipid material. Therefore, there is interest in the lipids of homopterans and how they obtain/synthesize and accumulate lipids (Strong, 1963; Brown, 1975; Febvay et al., 1992; Dillwith et al., 1993; Buckner, 1993).

The cuticular lipids of *B. argentifolii* nymphs (Buckner et al., 1999) and adults (Buckner et al., 1994; Nelson et al., 1994) have been characterized, but the identification and quantification of the internal lipids of whiteflies have not been determined. Some insects of three insect orders have been shown to synthesize linoleic acid de novo (Cripps et al., 1986), including the homopteran phloem-feeding pea aphid, *Acyrthosiphon pisum* (de Renobales et al., 1986). For whiteflies, linoleic acid as an essential or nonessential fatty acid has not been determined. The assessment of the internal lipids as a source of nutrient reward is especially relevant to predatory insects and to specific parasitoids of whiteflies, since they consume their host from within. In this study, we report on the identification and composition of the fatty acids associated with the major internal lipid classes (triacylglycerols, free fatty acids, and phospholipids) in *B. argentifolii* nymphs. Comparisons are made to the fatty acids present in the internal lipids of adult *B. argentifolii*. Radiotracer studies were conducted with *B. argentifolii* adults to determine their ability to synthesize polyunsaturated fatty acids de novo and mass spectrometry analysis of fatty acid pyrrolidine derivatives was used to determine the double bond positions for unsaturated fatty acids.

MATERIALS AND METHODS

Materials

Silica (Porasil 125 Angstrom) was purchased from Waters Corp., Franklin, MA. High performance thin-layer plates (10 × 10 cm, E. Merck, silica gel 60) were purchased from EM Science, Gibbstown, NJ. [2-^{14}C] Acetic acid, sodium salt (54 mCi/mmol) was purchased from New England Nuclear, Boston, MA. Ecolite and BetaMax (liquid scintillation cocktails) were purchased from ICN Biomedicals, Costa Mesa, CA. Authentic lipid standards were purchased from Nu-Chek Prep, Inc., Elysian, MN. Tertiary-butylmethylether and pyrrolidine were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. The 10% HCl in methanol reagent was prepared in-house by bubbling gaseous HCl into redistilled methanol. Reactivials were purchased from Pierce, Rockford, IL. Other chemicals were of analytical grade.

Insects

Silverleaf whiteflies, *B. argentifolii*, Bellows and Perring (Homoptera: Aleyrodidae), were initially obtained from the USDA-ARS Western Cotton Research Laboratory, Phoenix, AZ. A colony was subsequently maintained on hibiscus plants in rearing cages. Colony cages were placed within a walk-in environmental chamber equipped with 485 W high-pressure sodium lamps on a 15-h light/9-h dark cycle at 28°C. To obtain feeding nymphs on cantaloupe, young plants seeded in small peat balls were placed within colony cages for 2–3 days. The whitefly egg-laden plants/leaves were removed from the colony cages and held in separate chambers with controlled temperature and lighting. For determination of number of insects per pooled sample, collected nymphs were accurately counted, whereas quantities of adults were weighed and the number of adults per pooled sample was estimated assuming an average weight of 20 μg/adult.

Lipid Extraction Procedures

B. argentifolii adults were collected in a collection jar placed atop a colony cage by illumination of the jar with light passed through transparent yellow film. The adults were chilled at –20°C for 30 min and then removed from the jar, weighed and stored at –80°C. Groups of adults (20–75 mg fresh wt.) were placed into a 20-ml glass vial. Prior to extraction of internal lipids, waxy particles and cuticular lipids were removed by suspending the adults in 10 ml of hexane for 1–2 min as previ-

ously described (Buckner et al., 1994). For extraction of internal lipids, 8–10 ml of $CHCl_3$/MeOH (2:1) was added to the vial containing the hexane-extracted adults, and the vial was sealed with a Teflon-lined screw cap. To homogenize (sonication) the adult tissues, the vial was placed in the water bath of an ultrasonic cleaner (Model FS 60, Fisher Scientific, Pittsburgh, PA) for 30 min. The $CHCl_3$/MeOH homogenate was filtered through glass wool, and rinsed with additional $CHCl_3$ into a 30-ml separatory funnel. Water was added, and after shaking, the $CHCl_3$ was removed and saved. The water layer was partitioned with additional $CHCl_3$. The combined $CHCl_3$ layers were washed once with water, and after evaporating the $CHCl_3$, the lipid residue was stored under argon at –20°C.

For each pooled sample (115–408 nymphs), third or fourth instar nymphs were removed from colony cage hibiscus leaves or cantaloupe leaves with 0.12-mm gauge pins. Nymphs were transferred to a small pre-weighed aluminum pan that was kept chilled on ice. After collecting approximately 50 nymphs, the pan was brought to room temperature and the pan re-weighed to obtain the weight of the nymphs. The nymphs were rinsed off the weighing pans with hexane into a 20-ml vial fitted with a Teflon-lined screw cap. The hexane extract containing the surface lipids was removed and discarded. The hexane-extracted nymphs were then suspended in 7–8 ml of $CHCl_3$/MeOH (2:1), and the internal lipids extracted by placing the sealed vial in sonicator bath for 45 min. The lipid homogenate was then transferred into a separatory funnel. Additional groups of weighed nymphs were homogenized in $CHCl_3$/MeOH (2:1) and added to the separatory funnel to constitute a pooled sample. The $CHCl_3$ layers were washed with water as described above for *B. argentifolii* adults.

Thin-Layer Chromatography

For TLC analysis of neutral lipids, fractions were dissolved in 500 µl of $CHCl_3$ and 5 µl spotted alongside wax ester, triacylglycerol, and fatty alcohol standards onto a 10 × 10 cm (150 µm) silica gel plate. The plate was developed in hexane/ethyl ether/formic acid (80:20:1). For visualization, the plate was charred by spraying with 5% H_2SO_4 in 95% ETOH and heating to 200°C. For TLC of phospholipids, silica gel plates (10 × 10 cm; thickness of 150 µm) were impregnated with boric acid (0.8% w/v in ethanol). Phospholipid fractions were dissolved in 500 µl of $CHCl_3$/MeOH (2:1) and 5 µl spotted on each silica gel plate with phosphatidylethanolamine (PE) and phosphatidylcholine (PC) standards. The plate was developed in $CHCl_3$/MeOH/ammonia/water (60:37.5:1:3) and charred for viewing.

Fractionation of Lipids

The internal lipids in whitefly extracts were separated into neutral lipids, free fatty acids, and phospholipids using a modification of a silica column procedure (Hamilton and Comai, 1988). For separation of internal lipids of a pooled sample of nymphs, silica was packed into a 5¼″ glass Pasteur pipet (0.5 cm i.d.) fitted with a small plug of glass wool. The length of the column bed was usually 1 cm for nymph samples and 1–3 cm for adult samples. The silica in each column was equilibrated by washing with 4 ml of hexane/*tert*-butylmethylether (*t*-BME) (96:4) followed by 12 ml of hexane. The internal lipids from each sample were dissolved in 1–2 ml of hexane, with heating if necessary, and applied to the column. An additional 8 ml of hexane, followed by 8 ml of hexane/*t*-BME (99.5:0.5) was added to the column to elute surface lipid contaminants. Neutral lipid components were then eluted from the column with successive 8 ml additions of hexane/*t*-BME (96:4), hexane/acetic acid (100:2), and *t*-BME/acetic acid (100:0.2). For polar lipids (phospholipid fractions), the column was eluted with combinations of *t*-BME/methanol/0.001 M ammonium acetate (pH 8.6). To the column was added successive 8-ml volumes of *t*-BME/MeOH/ammonium acetate (40:4:1), *t*-BME/MeOH/ammonium acetate (20:4:1), and *t*-BME/MeOH/ammonium acetate (5:4:1). TLC methods, as described above, were used to determine the purity of column fractions.

Hydrolysis and Formation of Fatty Acid Methyl Esters

The fatty acids of lipid fractions were obtained by alkaline hydrolysis. Triacylglycerol and phospholipid fractions from silica columns and the total internal lipids (radiolabeled and unlabeled) from adult *B. argentifolii* were transferred with either $CHCl_3$ or $CHCl_3$/MeOH (2:1) into 1-ml Reacti-vials and the solvent removed by evaporation with N_2. To each vial was added 40 µl of benzene and 760 µl of 5% KOH in methanol. The vials were sealed with a Teflon-lined screw cap and placed in an aluminum-heating block at 75°C for 3–4 h with occasional mixing. Reaction mixtures were cooled and transferred to a 30-ml separatory funnel. $CHCl_3$ rinses of the vial were added to the funnel. The alkaline solution was neutralized with the addition of ~700 µl of 1.0 M HCl. After shaking, the $CHCl_3$ layer was removed and saved. The water layer was extracted with additional $CHCl_3$. The combined $CHCl_3$ layers were extracted with additional water, then evaporated and the residue stored under argon at –20°C.

For formation of methyl ester derivatives, the free fatty acid fraction from the silica column, and the fatty acid samples resulting from alkaline hydrolysis of triacylglycerol and phospholipid column fractions, and other total lipid extracts, were dissolved in $CHCl_3$, and then transferred into 1-ml Reacti-vials. After evaporation of the solvent with N_2, 40 µl of benzene and 760 µl of 10% HCl in methanol were added to each vial. The vials were sealed with a Teflon-lined screw cap and placed in a heating block at 75°C for 1–2 h. Each cooled reaction mixture was transferred to a 30-ml separatory funnel and $CHCl_3$ rinses of the vial were added to the funnel. The $CHCl_3$-containing solution was partitioned against water. The $CHCl_3$ layer was evaporated and the fatty acid methyl esters (FAME) were stored under argon at –20°C.

Gas Chromatography and Quantification of Lipid

The FAME were separated and analyzed by capillary gas chromatography (CGC). CGC analyses were performed using a Hewlett-Packard Model 5890 GC equipped with a split/splitless injector set at 260°C, a SP-2380 capillary column (30 m × 0.25 mm i.d.; Supelco, Bellefonte, PA), a flame ionization detector (FID), and helium as carrier gas. The column oven temperature was held at 110°C for 1 min, increased to 260°C at 10°C/min, and then held at 260°C for 1.5 min. The quantities of FAME were determined by using integrated peak area data from the FID response. Conversions of peak areas to ng quantities for FAME were made by using nonlinear regression slope data for increasing amounts (0.39–100 ng) of the authentic methyl esters of 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, and 20:0 fatty acids. Prepared samples for CGC-FID analysis contained an internal standard of 100 ng of palmityl acetate for normalization of the data. Confirmation of the identity of fatty acids as their methyl esters was conducted by CGC-mass spectrometry analysis as previously described (Buckner et al., 1999).

Adult Feeding Experiments With 2-^{14}C Acetate

For a typical experiment, the adult whitefly feeding media was prepared by transferring 500 µl (58 µCi) of the radiolabeled acetate stock solution ([2-^{14}C]-acetate in EtOH) into a 1.5 ml polypropylene microfuge tube. The EtOH was evaporated and replaced by 1.25 ml of sterile 15% sucrose. Each membrane apparatus consisted of thinly stretched Parafilm M® covering the 1-cm diameter opening of a 0.5-ml polystyrene conical capsule. The membrane-attached end of each of five capsules was fitted into a tight fitting hole in the center area of the lid of a 15-cm diameter × 6-cm high polystyrene Petri dish. The dish and lid were painted black to exclude light. *B. argentifolii* adults were removed from hibiscus leaves in colony cages and transferred into the membrane/capsule-fitted Petri dish. Through a hole made in the tapered bottom of the capsule, 0.25 ml of radiolabeled media was transferred onto the inner surface of each capsule membrane. As previously described (Buckner et al., 2002), an airtight plastic cabinet was used to maintain the membrane/capsule-fitted Petri dish at a relative humidity of 98–100% and yellow light was

used to lure adults to the five membrane surfaces. After incubation at 25°C for 48 h, the membrane/capsule-fitted Petri dish/lid was chilled in a –20°C freezer for 30 min to incapacitate the feeding *B. argentifolii* adults. Adults were individually removed from the surfaces of the membranes and from the bottom of the Petri dish and placed into an ice-chilled 20-ml glass vial. Methods for removal of surface lipids, the homogenization, and recovery of radiolabeled internal lipids with $CHCl_3$/MeOH and the hydrolysis/esterification of fatty acids from adult whiteflies were as described above.

Radioactivity in lipid extracts was determined by liquid scintillation counting (LSC). Aliquots of lipid samples dissolved in $CHCl_3$ were transferred into 7-ml glass vials. The $CHCl_3$ was evaporated and replaced by 6.5 ml of BetaMax LSC cocktail. Radiolabeled FAME were separated and analyzed by HPLC using a Waters Model 2695 Separations Module (Waters Corporation, Milford, MA). An Inertsil ODS-3 reverse phase column (100 × 2.1 mm, 3-µm particle size; MetaChem Technologies Inc., Torrence, CA) was used and components were eluted from the column with a solvent gradient (water/CH_3OH (25:75) to 100% acetone in 50 min) at a flow rate of 0.25 ml/min. Column fractions (42 µl) were collected into 7-ml LSC vials and mixed with 6.5 ml of Ecolite LSC cocktail. Radioactivity in LSC vials was measured using a Packard Model 2300TR liquid scintillation analyzer (Packard Instruments, Co., Meriden, CT) at a counting efficiency of 97% for ^{14}C. Following HPLC separation, unlabeled FAME standards were detected using a Sedex (Model 55) evaporative light scattering detector (ELSD) (SEDERE, Alfortville, France) at 28°C and N_2(g) pressure at 2.1 bar.

Fatty Acid Double Bond Location

For determination of double bond location, the fatty acids from the internal lipids of *B. argentifolii* were analyzed by GCG-MS as their pyrrolidine derivatives. The fatty acid methyl esters, obtained from hydrolysis and esterification of the internal lipids of approximately 2,000 *B. argentifolii* adults (as described above), were transferred into a 5-ml vacuum hydrolysis tube with $CHCl_3$. After evaporation of the $CHCl_3$ under a stream of N_2(g), the tube was flushed thoroughly with argon. After addition of 1 ml pyrrolidine and 0.1 ml glacial acetic acid, the tube was sealed under vacuum. The reaction mixture was heated at 100°C for 1 h. After cooling to room temperature, the reaction mixture was transferred into a separatory funnel and neutralized with about 5 ml of 20% HCl. The aqueous fraction was partitioned twice against 15 ml $CHCl_3$. The combined $CHCl_3$ was backwashed with 5 ml of water. The volume of $CHCl_3$ was reduced to less than 10 ml on a rotary evaporator and then dried over anhydrous $MgSO_4$. Without taking it to dryness, the sample of fatty acid pyrrolidides was adjusted to the desire volume and 1-µl aliquots were analyzed using a Hewlett-Packard 5970 GC-MS equipped with a Supelco SP-2380 capillary column (30 m × 0.25 mm × 0.2 µm film thickness) and splitless injector at 260°C. The oven temperature was held at 50°C for 2 min, then ramped at 25°C per minute to 260°C and held 10 min. Helium flow rate was 1 ml/min.

RESULTS

Extraction and Separation of Lipid Classes

After removing the majority of the surface lipids with hexane, the internal lipids of nymphs and adults were efficiently extracted into chloroform/methanol (2:1) with sonication. High performance thin-layer chromatography (HPTLC) of portions of the $CHCl_3$/MeOH extracts was used to determine that the major neutral lipids were triacylglycerols (data not shown). Adult samples contained small quantities of long-chain aldehydes and alcohols that presumably resulted from the incomplete removal of waxy particles from the adult cuticle. HPTLC also revealed that the major polar lipids were the phospholipids, PE and PC. Small columns packed with silica gel were used to successfully separate the internal lipids into lipid classes. Lipid extracts dissolved in hexane were applied to the column and initially eluted with hexane and then hexane/*t*-BME (99.5:0.5) to remove traces of the

surface lipid contaminants, hydrocarbons and wax esters. The major lipid class of the internal lipids of nymphs and adults, TAG, was completely eluted with the hexane/*t*-BME (96:4) solvent mixture. Hexane with 2% acetic acid was the solvent system that removed the small quantities of free fatty acids from the silica column. No TLC-detectable lipids were eluted from the columns using the more polar solvent mixture: *t*-BME/acetic acid (100:0.2). Polar lipids were eluted with mixtures of *t*-BME, methanol, and 0.001 M ammonium acetate (pH 8.6). HPTLC of the fraction eluted with *t*-BME/MeOH/ammonium acetate (40:4:1) revealed no polar lipids in nymphal extracts and barely detectable amounts of an unknown fraction in adult extracts. Elution with *t*-BME/MeOH/ammonium acetate (20:4:1) followed by *t*-BME/MeOH/ammonium acetate (5:4:1), yielded the PE and PC fractions, respectively.

Quantities of Fatty Acids

Fatty acids were recovered from the TAG, PE, and PC fractions following alkaline hydrolysis. Methyl esters of the fatty acids were formed by reaction with methanolic HCl, and the fatty acid methyl esters (FAME) were identified and quantified by CGC. As shown in a typical CGC-FID chromatogram of a nymph sample (Fig. 1A), lipid classes from both *B. argentifolii* nymphs and adults contained variable distributions of eight fatty acids: the saturated fatty acids, myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), arachidic acid (20:0); the monounsaturated fatty acids, palmitoleic acid (16:1), oleic acid (18:1); and the polyunsaturated fatty acids, linoleic acid (18:2), linolenic acid (18:3). FAME with retention times longer than 20:0 and 18:3 were not detected in any of the nymph and adult samples analyzed by CGC-FID using the fatty acid analysis column.

The quantities of total lipid and amounts within each lipid class were variable for each of the two pooled groups of third-instar *B. argentifolii* nymphs and the two groups of fourth-instar nymphs (Table 1). Variability was, in part, due to the observed differences in the size of nymphs removed from

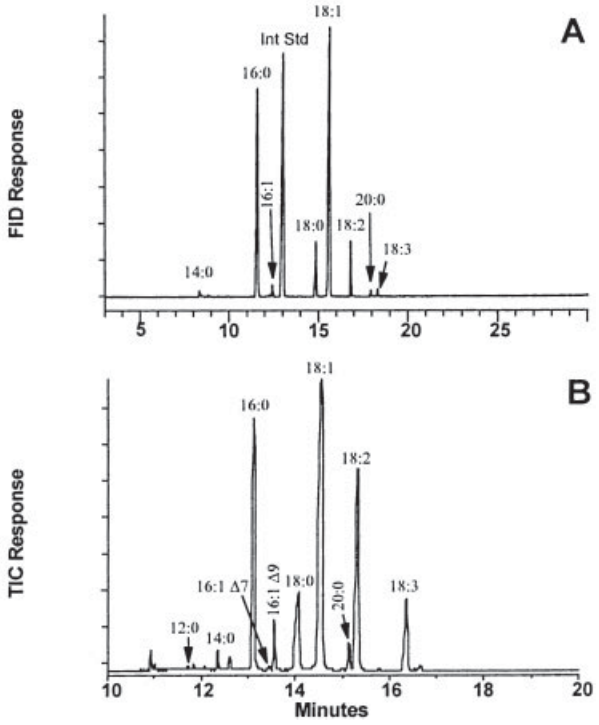

Fig. 1. **A:** A CGC-FID chromatogram of FAME from the TAG fraction from 115-pooled fourth instar nymph sample. The chromatogram represents FID response for FAME from 0.2 nymph equivalents. CGC analysis showed eight peaks that were identified (in order of elution) as: myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), arachidic acid (20:0), and linolenic acid (18:3). Int. Std. = Internal standard detector response for 100 ng of palmityl acetate. **B:** A CGC-MS chromatogram of pyrrolidine derivatives of internal lipid fatty acids from a pooled sample of 2,030 adults. The chromatogram represents total ion current (TIC) response for fatty acid pyrrolidides from 4.0 adult equivalents.

leaves. Overall, the quantities of internal lipids from fourth-instar nymphs were approximately 10 times the amounts from third-instar nymphs and these differences correlated with the size and weight differences between groups and instars. Although their quantities were variable, the distributions of fatty acids among the lipid classes for the groups of third- and fourth-instar nymphs were quite similar. The mean percentage distributions (n = 4) of fatty acids in the TAG, FFA, PE, and PC fractions from third- and fourth-instar nymphs

TABLE 1. Quantities and Distribution of Fatty Acids in Lipid Classes of Nymphs and Adults

Number of pooled insects[a]	Average fresh wt. (μg/nymph)	Fatty acids (pmol/nymph or adult)[b] TAG	FFA	PE	PC	Total
3rd Instar nymphs						
408	7.4	345.6	13.0	28.8	31.7	419.1
216	8.4	595.4	3.5	17.4	66.6	682.9
4th Instar nymphs						
142	18.6	2,934.2	36.3	74.8	188.2	3,233.5
115	28.4	3,524.8	24.3	249.9	496.8	4,295.8
% Distribution[c]		85.6 ± 4.1	1.3 ± 1.2	4.4 ± 2.3	8.7 ± 2.5	
Adults[d]						
800		811.4	—	430.7	93.0	1,335.1
3,750		896.8	28.0	409.4	291.8	1,626.0
400[e]		1,616.1	12.6	660.4	450.5	2,739.6
600		1,252.8	31.3	597.7	273.5	2,155.3
% Distribution[c]		58.3 ± 2.3	1.2 ± 0.6	27.3 ± 3.6	13.5 ± 4.8	

[a]Number of insect equivalents for each nymph or adult total lipid extract applied to a silica column for lipid class fractionation.
[b]Picomoles of fatty acids for each lipid class were determined by totaling the amounts of fatty acids as quantified by CGC-FID analyses.
[c]Values represent the means (± S.D.) for the mole percents of lipid classes for each of the 4 groups of pooled 3rd and 4th instar nymphs and for each of the 4 groups of pooled adults.
[d]Number values for pooled adult groups (800, 3,750, 400, and 600) were calculated from the group weights of 16, 75, 8, and 12 mg, respectively, and from an average fresh wt. of 20 μg/adult.
[e]From an original pooled group of 2,550 adults (51.1 mg).

were 85.6, 1.3, 4.4, and 8.7, respectively. These amounts were compared to the internal lipids from *B. argentifolii* adults. The quantities of fatty acids in adult lipid classes were somewhat variable and the total amounts (pmol/adult) for each pooled group were less than the amounts from last (fourth) instar nymph groups. Just like the groups of nymph samples, the distributions of fatty acids for each lipid class were quite similar. The amount of total fatty acids in the TAG fractions of the adult groups averaged 58.3 ± 2.3% (n = 4), but when compared to nymphs, the adults groups had a larger percentage of fatty acids in the PE (27%) and PC (13%) fractions (Table 1). For both nymphs and adults, FFA was a minor lipid class and, within groups, the amounts were variable.

To check for complete recovery of lipid from the silica columns, the total quantity (pmol/adult) of FAME from the summed TAG, FFA, PE, and PC column fractions of the 400-insect equivalent sample (see Table 1) was compared with FAME analysis from the hydrolysis/esterification of an additional 400-adult equivalent amount of the same adult extract (originally from a pool of 2,550 adults). The pmol/adult total for the unfractionated sample did not exceed the total of FAME for the combined TAG, FFA, PE, and PC column fractions (data not shown). Thus, it was apparent that the solvent mixtures used to fractionate the lipid extracts had eluted all internal lipid classes from the column.

Distribution of Fatty Acids in Lipid Classes

The mean percent composition was determined for the fatty acids from the TAG, PE, and PC fractions of both *B. argentifolii* nymphs and adults. The fatty acid distributions for the pooled groups of third- and fourth-instar nymphs shown in Table 1 were very similar, and the percent composition data for each lipid class were combined and the results shown in Figure 2. For the TAG lipid fraction, the distribution of fatty acids was similar for both nymphs and adults. The major fatty acid constituents for nymphs were 18:1 (48%) and 16:0 (33%), with lesser amounts of 18:0 and 18:2 (Fig. 2A). Only trace amounts were detected for 14:0, 20:0, and the unsaturated fatty acids, 16:1 and 18:3. For adult TAG, the percentage composition values for 18:1 and 16:0 were 58 and 29%, respectively.

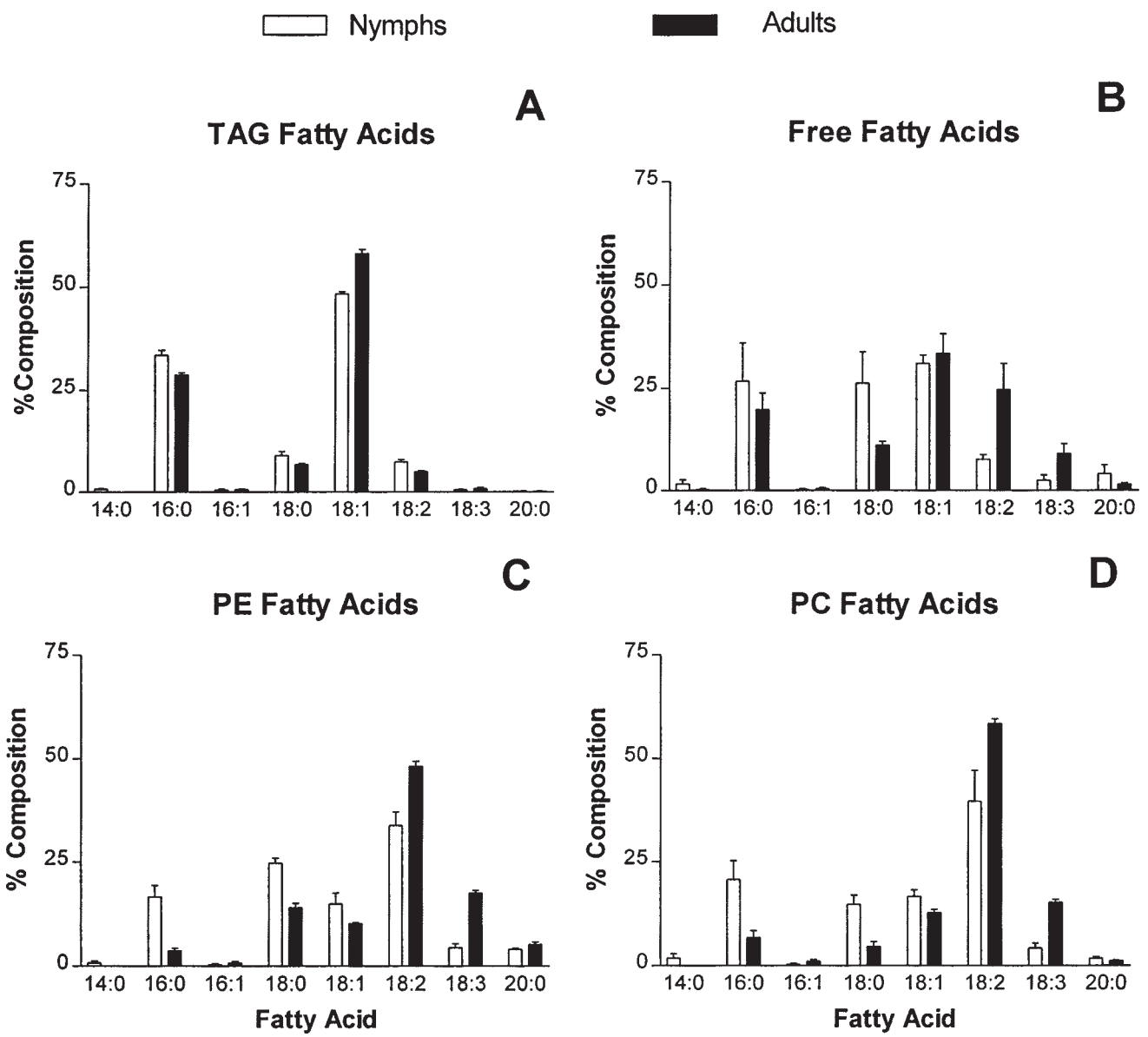

Fig. 2. The mean percentage composition of fatty acids from each lipid class for nymph (third and fourth instars combined) and adult samples shown in Table 1 (n = 4). The lines above the bars indicate standard deviation.

The percent distribution of fatty acids from the phospholipids (PE and PC) of nymphs and adults were different when compared to the TAG fraction. For the PE fraction from nymphs, the major fatty acids were 18:2 (34%), 18:0 (25%), 16:0 (17%), and 18:1 (15%), with lesser quantities of 18:3 and 20:0 (Fig. 2C). For PE from adults, the polyunsaturated fatty acids 18:2 (48%) and 18:3 (18%) were the major constituents with lesser amounts of 18:0, 18:1, 20:0, and 16:0, in decreasing order of abundance. The fatty acid distributions from the PC fractions of nymphs and adults (Fig. 2D) were similar to those for the PE fractions. The major constituent for the fatty acids from the nymph PC fraction was 18:2 (40%), and for the adult PC fractions, 58% of the fatty acid mixture was the 18:2 constituent. The amounts of FFA in extracts from nymphs and adults were relatively small and the distribution percentages were more variable when compared to the other lipid classes. For the nymphs,

the major FFA constituents were 18:1, 16:0, and 18:0, whereas for the adults, 18:1, 18:2, and 16:0 were the major acids (Fig. 2B).

[2-^{14}C] Acetate Incorporation Into Lipids

Radiolabeled acetate, as a biosynthetic precursor of fatty acids, was successfully incorporated into the internal lipids of feeding *B. argentifolii* adults. Quantities of [2-^{14}C] acetate were added to media (0.046 µCi/µl) contained within an artificial membrane apparatus. In repetitive feeding experiments, the quantities of radiolabel in $CHCl_3$/MeOH extractable lipids from 225–750 *B. argentifolii* adults after 48 h of feeding varied between 1,400 and 3,800 dpm/adult. Separation and purification of lipid classes by TLC and open-column silica gel chromatography indicated that 60–70% of the labeled lipids were associated with the triacylglycerols, 15–20% in the phospholipids fractions, and the remainder in other neutral lipid fractions (data not shown). This distribution of radiolabel among internal lipid classes is similar to the percentage distribution of unlabeled lipid classes for adult *B. argentifolii* reported in this study (Table 1).

The distribution of radiolabel in fatty acids from the total lipids extracted from *B. argentifolii* adults that had fed on [2-^{14}C] acetate-containing media was determined by HPLC following alkaline hydrolysis and formation of methyl ester derivatives. A reverse-phase column and water/MeOH/acetone solvent gradient system was used to separate the various FAME components including the base-line resolution of the 18:2 FAME (Fig. 3). A typical histogram generated from 10-sec fractions of the HPLC column eluent and subsequent ^{14}C assay by LSC showed that the majority of the radioactivity (88% of injected radiolabel) was incorporated into peaks with retention times identical to FAME of authentic 18:3, 16:1, 18:2, 16:0, 18:1, 18:0, and 20:0 (Fig. 3). For the monounsaturated fatty acids, the peak corresponding to 18:1 contained 51.8% of the radioactivity and 1.9% was associated with the peak for 16:1. The distribution of radioactivity for the polyunsaturated peaks for 18:2 and 18:3 was 4.9 and 0.9%, respectively. The remainder of the ^{14}C was distributed among the saturated fatty acids, 16:0 (29.8%), 18:0 (9.9%), and 20:0 (0.7%).

Double Bond Positions for Unsaturated Fatty Acids

The position of double bonds in the fatty acids of *B. argentifolii* was determined by CGC-MS analysis of the pyrrolidine derivatives of the mono-, di-, and tri-unsaturated fatty acids. The pyrrolidides of the fatty acids from pooled samples of *B. argentifolii* adults were clearly resolved by the capillary column (Fig. 1B). For the mass spectra of an unsaturated component, if an interval of 12 atomic mass units is observed between the most intense peaks of clusters of fragments containing n and n-1 carbon atoms of the acid moiety, the double bond occurs between carbons n and n+1 in the molecule (Andersson and Holman, 1974). The mass spectrum for the 18:1 peak was consistent for the pyrrolidine derivative of octadec-9-enoic acid (oleic acid) with a molecular ion at *m/z* 335, a base peak at *m/z* 113 for the McLafferty rearrangement of all fatty acid pyrrolidides and diagnostic fragments ions 12 amu apart at *m/z* 196 and *m/z* 208 for a double bond at position 9 (Δ^9).

For the peak of 18:2, a molecular ion at *m/z* 333 and characteristic series of fragment ions at *m/z* 196, 208 and *m/z* 250, 262 for double bond positions at 9 and 12, respectively, are consistent for the pyrrolidine derivative of octadec-9,12-dienoic acid (linoleic acid). A molecular ion at *m/z* 331 and an overall mass spectral fragmentation pattern that was identical to the mass spectrum for the pyrrolidide of authentic octadec-9,12,15-trienoic acid strongly suggested that the 18:3 peak from *B. argentifolii* adult fatty acids was linolenic acid. A molecular ion at *m/z* 307 and diagnostic fragment ions characterized two positional isomers of 16:1 with a double bond at Δ^9 (palmitoleic acid) and Δ^7.

DISCUSSION

The fatty acids of an insect function as a form of energy storage and as components of cellular and subcellular membranes (Gilbert, 1967; Downer,

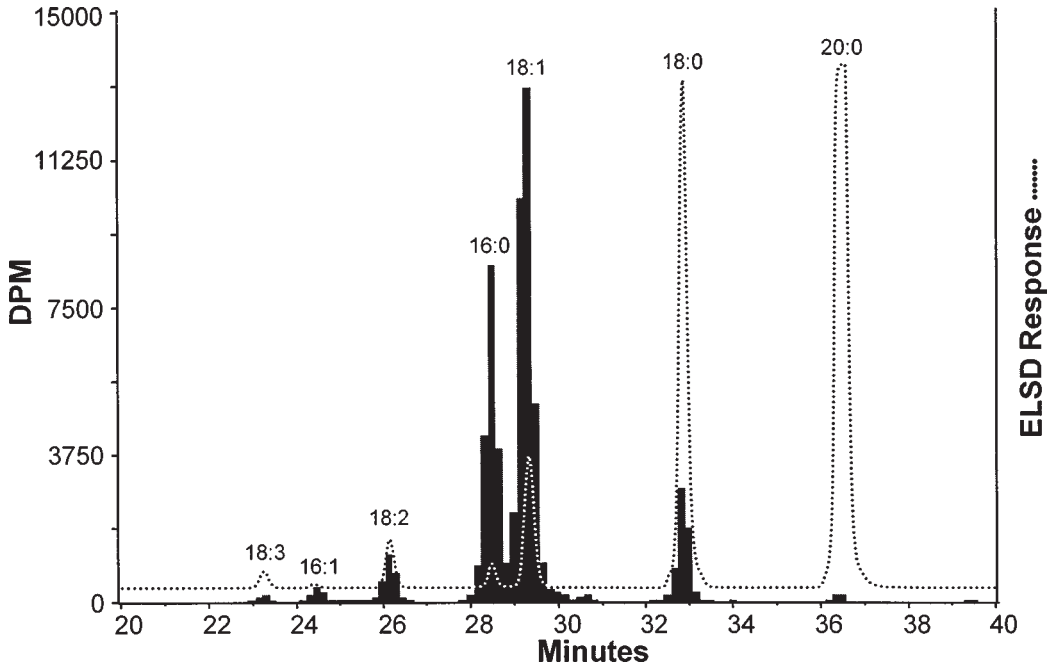

Fig. 3. Distribution of radiolabel in the fatty acids from the internal lipids of 750 *B. argentifolii* adults fed artificial media containing [2-^{14}C] acetate. A portion of the labeled fatty acids (85,000 dpm and 25 insect equivalents) as their methyl esters, were separated by HPLC. Fractions (10 sec each) were collected into counting vials and assayed for radioactivity (DPM). Unlabeled FAMEs of authentic standards (10 µg each) were detected using an evaporative light scattering detector (ELSD) and a HPLC chromatogram (· · · · · ·) is shown on the ^{14}C histogram. Injection of samples, chromatography by reverse phase HPLC and radioactivity assays were performed as described in Materials and Methods.

1985; Stanley-Samuelson and Dadd, 1983; Stanley-Samuelson et al., 1988). In most insects, the majority of the fatty acids are associated with triacylglycerols that are usually stored within fat body cells (Kilby, 1963; Downer, 1985). Fatty acids are also associated with the phospholipids and sterol ester components of biomembranes. Furthermore, fatty acids in insect internal lipids are essential to the nutritional needs for predacious insects (Cohen, 1995). In our previous studies of predators and parasitoids of *B. argentifolii* nymphs, the internal contents were usually consumed in their entirety, leaving only the nymphal exocuticle. Predacious insects that ingest only the internal contents of their prey select nutrient-rich food unburdened by the indigestibility of cuticular structures (Cohen, 1995). Most natural enemies of whiteflies that use extra-oral digestive processes have effective mouthparts for probing, delivery of digestive juices and uptake of liquefied nutrients. The digestive enzymes are hydrolases that include triacylglycerol lipases for digesting the storage triacylglycerols localized in fat body tissues and phospholipases for digesting the phospholipids in cellular and subcellular membranes (House, 1974; Turunen 1979; Cohen, 1995).

In this study, we provide results on the separation and quantification of internal lipid classes, and fatty acid composition of the silverleaf whitefly, *B. argentifolii*. Groups of feeding adult whiteflies were removed from hibiscus leaves. For third- and fourth-instar nymphs, preliminary results were compared for feeding nymphs removed from hibiscus to those from cantaloupe and no apparent differences were observed. Therefore, the results presented in this study were from nymphs reared on cantaloupe. From the average fresh weight of collected third- and fourth-instar nymphs (see

Table 1) and an average fresh weight of 20 μg for each adult, the amounts of internal lipids as a percent of body weight were estimated at 1.5–2.5, 4–5, and 2–4% for third-instar nymphs, fourth-instar nymphs, and adults, respectively. The estimated 4–5% amount for fourth-instar nymphs is in the same range as reported for another homopteran, the pea aphid, *Acyrthosiphon pisum* (Febvay et al., 1992). In Table 1, the pooled group of 115 fourth-instar nymphs represents fully mature nymphs that have attained their maximum weight and amount of stored lipid for subsequent pupal and adult transformations. Nymphs of this group had 1.5 to 3.0 times the amounts of total fatty acids as adults, mostly as larger amounts of fatty acids in the TAG fraction (Table 1). The lower amounts of TAG fatty acids for the adult groups could have arisen from metabolism and conversion into the increased amounts of phospholipids during adult development and/or utilization for flight energy and reproductive processes.

For homopterans, different insects can have characteristic fatty acid profiles (Fast, 1970). Characteristic of aphids (Homoptera: Aphidoidae) are the very high quantities of 14:0 associated with the TAG (Fast, 1970; Febvay et al., 1992), and the Coccids have very high proportions of the short-chain acids, 10:0 and 12:0 (Fast, 1970). In contrast, ceropids and cicadellids have low proportions of short-chain fatty acid components and large amounts of 16:0, 18:1 and 18:2 (Fast, 1970). Similarly, the fatty acids of internal lipids from the whitefly (Homoptera: Aleyrodidae), as characterized in this study, were mainly 16- and 18-carbon constituents (Fig. 1). The chain-length distribution of triacylglycerols from nymphs showed high proportions of 18:1 (48%) and 16:0 (33%) (Fig. 2A). In this study, the fully mature fourth-instar nymph group represents the whitefly stage of development for predators/parasitoids that has the maximum quantity of nutrient. For the pooled group of 115 fourth-instar nymphs, the quantities of the various fatty acids were listed for each lipid class to illustrate which lipid class was the major source for each fatty acid component (Table 2). Over 90% of the monounsaturated 18:1 and saturated 16:0 fatty acids were associated with the TAG fraction.

Triacylglycerol characterization has been reported for other whitefly species. Chloroform or chloroform:methanol extracts of bulk samples of whole adult *Bemisia tabaci* and *Trialeurodes vaporariorum* yielded lipids that were reported to consist mainly of triacylglycerols (65–89%) as components of the surface lipids plus wax particles (Baker and Jeffree, 1981; Byrne and Hadley, 1988). Following alkaline hydrolysis of the triacylglycerols, the liberated fatty acids were identified as a 2:1 mixture of stearic (18:1) and palmitic (16:0) acids and the probability that the major compound was glyceryldioctadecenylhexadecanoate (palmitoyl dioleoyl glycerol; POO) was confirmed by mass spectrometry (Baker and Jeffree, 1981). More recent studies have concluded that the triacylglycerols were not components of the surface lipids of *B. tabaci* and *T. vaporariorum* adults (Nelson et al., 1994; Buckner et al., 1994) and the presence of TAG in the lipid

TABLE 2. Quantities of Constituent Fatty Acids for Each Lipid Class From a Pooled Group of 115 Fourth Instar Nymphs

	pmol/nymph[a]							
Lipid class	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
TAG[b]	15.2	1,224.2	37.6	229.5	1,755.0	229.5	29.7	4.1
FFA	0.0	7.2	0.2	5.5	8.8	1.4	0.3	0.9
PE	0.0	21.5	1.7	63.2	28.1	106.3	17.5	11.6
PC	0.0	47.1	3.5	40.5	63.2	300.1	37.2	5.2
Total	15.2	1,300.0	43.0	338.7	1,855.1	637.3	84.7	21.8

[a]The quantities of fatty acids for each lipid class were determined by using integrated peak area data from CGC-FID response of each fatty acid as its methyl ester derivative. Conversions of peak area to pmol amounts were obtained from standard curves for authentic FAME as described in Materials and Methods.
[b]Values for TAG fatty acids were obtained from CGC-FID response shown in Figure 1A.

extracts reported in the earlier studies probably resulted from inadvertent extraction of adult internal lipids.

The involvement of 18:1 and 16:0 as major constituents of the TAG fractions (> 80%; Fig. 2A) was confirmed by HPLC-MS analyses of intact TAG fractions from adult samples. The major compounds in the adult TAG fraction (> 90%) were identified as triacylglycerols with different combinations of mainly the 18:1 and 16:0 fatty acid constituents: palmitoyl dioleoyl glycerol (POO), dipalmitoyl oleoyl glycerol (PPO), palmitoyl stearoyl oleoyl glycerol (PSO), trioleoyl glycerol (OOO), and stearoyl dioleoyl glycerol (SOO) in decreasing order of abundance (Buckner and Hagen, unreported data). In contrast, more than 95% of the triacylglycerols from adult greenhouse whiteflies (*T. vaporariorum*) were characterized as POO (Baker and Jeffree, 1981).

After TAG, the phospholipid fractions PE and PC were the next most abundant lipid components (Table 1; Figs 2C and D). In insects, PE and PC are the major phospholipids within the body, and account for over 70% of the total phospholipid complement in most species examined (Fast, 1970; Downer, 1985). For the total lipid fatty acids from *B. argentifolii* nymphs, the mean percentage distribution for fatty acids from PC fractions was 8.9%, and was approximately twice the value of the PE fractions (PC/PE = 1.97). Adult whiteflies had higher distributions of their total fatty acids derived from phospholipids (~40%); in contrast to nymphs, PE amounts were greater than PC (PC/PE = 0.49). For other homopterans, PE was the major phospholipid in several aphid species (Fast, 1966; Febvay et al., 1992) and a spittlebug (Fast, 1966). As shown in Table 2 for the mature fourth-instar nymphs, 65% of the 18:2 polyunsaturated fatty acid was typically associated with the phospholipid fractions. For both PE and PC fractions, 18:2 was the major fatty acid (33.9 and 39.6%, respectively, for nymphs; 48.2 and 58.5%, respectively, for adults: see Fig. 2C and D). The phospholipid fatty acid composition for another homopteran, the cicada *Tibicen dealbatus* has been reported. For the phospholipids from adult *T. dealbatus*, large proportions of the fatty acid components (about 80%) were equally distributed between 18:2 and 18:1, but only small quantities of 18:3 were detected (Stanley-Samuelson et al., 1990).

Nutritional and biochemical studies have determined that linoleic acid (18:2) is an essential fatty acid for some insects and, for other insect species, an ability to synthesize linoleic acid has been documented (Downer, 1978; Dwyer and Blomquist, 1981; Blomquist et al., 1982; Cripps et al., 1986). For a homopteran, the pea aphid, *A. pisum*, a radiolabeled lipid precursor was incorporated into linoleic acid in a time-dependent manner (de Renobales et al., 1986). Whiteflies, like aphids, are phloem feeders, and it was suggested that their food supply does not provide them with adequate levels of linoleic acid (de Renobales et al., 1986). Also, whiteflies and aphids possess intracellular symbiotic bacteria that are thought to carry out some of the metabolic processes necessary for growth and reproduction of the host insect (Byrne and Bellows, 1991; Dillwith et al., 1993). Although difficult to rule out the involvement of symbiotes in homopteran lipid metabolism, biosynthetic studies with the pea aphid strongly suggest that linoleic acid is synthesized by insect tissues (de Renobales et al., 1986). The ability of homopterous insects to synthesize polyunsaturated fatty acids was confirmed as a result of our feeding studies with *B. argentifolii* adults and radiolabeled acetate. Chromatographic analysis of the FAME from radiolabeled internal lipids and assay for ^{14}C demonstrated the presence of a resolved radioactive component that co-chromatographed with an 18:2 (linoleic acid) standard (Fig. 3). Furthermore, the percentage distributions for the other labeled fatty acids were very similar to the distribution of identified and quantified unlabeled adult fatty acids shown in Figure 2A–D. Any evidence for whitefly symbiotic bacteria involvement in the radiolabeled incorporation studies was not established.

Further evidence that the 18:2 and 18:3 components were indeed linoleic acid and linolenic acid, respectively, was provided by mass spectral analysis of the *B. argentifolii* unsaturated fatty acids as their pyrrolidine derivatives. Pyrrolidine de-

rivatives of unsaturated fatty acids give diagnostic mass spectra for the location of double bonds without methods of chemical modification involving derivatization at the double bond (Andersson and Holman, 1974; Andersson, 1978; Christie et al., 1986). This method was applied to the pyrrolidine derivatives for the fatty acids of the internal lipids of *B. argentifolii* adults. The double bond positions for 18:1, 18:2, and 18:3 were established at positions Δ^9, $\Delta^{9,12}$, and $\Delta^{9,12,15}$, respectively, and the mass spectral fragmentation patterns for the *B. argentifolii* fatty acids were identical to the mass spectra for the pyrrolidine derivatives of the corresponding authentic oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) standards (data not shown). Thus, these findings clearly demonstrate the ability of whiteflies to synthesize polyunsaturated fatty acids de novo.

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